MYCOTAXON

ISSN (print) 0093-4666 (online) 2154-8889 © 2016. Mycotaxon, Ltd.

January–March 2016—Volume 131, pp. 103–110

http://dx.doi.org/10.5248/131.103

Phylogenetic placement of the secotioid fungus *Araneosa columellata* within *Agaricus*

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Abstract —Recently collected material was used to evaluate the phylogenetic position of the secotioid fungus *Araneosa columellata*. We amplified the nuclear ribosomal RNA gene (ITS1, ITS2, and LSU) and found *Ar. columellata* to be a member of the genus *Agaricus* (*Agaricaceae*, *Agaricales*), necessitating its transfer to that genus as *Agaricus columellatus* comb. nov. A detailed description is provided along with illustrations, and a lectotype is designated.

Key words — *Basidiomycota*, molecular phylogeny, Sonoran Desert, taxonomy

Introduction

DNA-based studies demonstrate multiple origins of gastroid (angiocarpic forms) and secotioid (stipitate angiocarpic forms often with reduced lamellae) species from within agaricoid (mushroom-forming) groups in the fungal phylogeny (Hibbett et al. 1997, Vellinga et al. 2003, Lebel & Syme 2012). Modern phylogenetic taxonomy (Queiroz & Gauthier 1992) stipulates that taxa be circumscribed as monophyletic clades, and this has necessitated the taxonomic reevaluation of these fungi. As a result, many familiar gastroid or secotioid taxa have been transferred into genera once reserved solely for agaricoid forms. For example, *Endoptychum agaricoides* was transferred to *Chlorophyllum* as *C. agaricoides* (Vellinga 2002), while *Endoptychum depressum* was transferred to *Agaricus* as *Ag. inapertus* (Vellinga et al. 2003).

Long (1941) described the monotypic secotioid genus *Araneosa* (type species: *Araneosa columellata*) within *Arachniaceae* (Coker & Couch 1928) from material collected at two localities in southern Arizona. Species within *Arachnion*, such as *A. album,* are superficially similar to *Ar. columellata*; however, *Araneosa* is distinguished primarily by its stipitate habit and the presence of a distinct columella within the gleba (Long 1941). Molecular phylogenetic studies have been needed to determine the taxonomic position of *Ar. columellata* (Moreno et al. 2007).

Several specimens of *Ar. columellata* collected by Long in the 1930–40s are deposited in institutional collections throughout the United States and Canada (BPI, FH, ILL, NY, NCU, UBC), many of which represent splits of the original type material (isotypes) in various stages of disintegration. An exceptionally well-preserved isotype (Fig. 1E) was located at the University of Illinois Herbarium, and this specimen is selected as the lectotype here. Specimens of this age are typically not desirable for DNA sequencing (Osmundson et al. 2013), and despite the need for new material, collections of this fungus have rarely been made (Esqueda 1998). On a recent excursion to the southwestern United States several fruiting bodies of *Ar. columellata* were collected. These specimens were morphologically identical to those described in the protologue (Long 1941) and yielded sufficient genomic DNA for Sanger sequencing of the nuclear ribosomal RNA gene (ITS1, ITS2, and LSU). This allowed us to examine the phylogenetic placement of *Ar. columellata* within the *Agaricales*.

Materials & methods

Original descriptions were made from material collected in the field, now deposited in the Fungal Collection of the Bell Museum of Natural History Herbarium at the University of Minnesota (MIN). An Olympus BH2 conventional bright field microscope was used in gathering the micro-morphological data on material first infiltrated with 95% aqueous ethanol and then mounted in water or 3% aqueous KOH. All measurements were made at 1000× using an ocular reticle with units calibrated by an optical micrometer. Spore statistics include: *x*, the arithmetic mean of the spore length by spore width (± SD) for n spores measured; Q, the quotient of spore length and spore width in any one spore, indicated as the range of variation in n spores measured; Q_m the mean of Q values. Classical descriptive methodologies follow those published previously for angiocarpic fungi (Miller & Miller 1988), while color descriptions follow the system outlined in the Methuen Handbook of Colour (Kornerup & Wanscher 1967). Standard abbreviations for herbarium collections follow Index Herbariorum (Thiers 2016).

Genomic DNA was extracted following standard protocol of the Qiagen's DNeasy Kit, after first grinding glebal tissue and spores with a micro-pestle and then incubating this material in the kit buffer for 10 min. at 65°C. To amplify nuclear ribosomal RNA genes (ITS1, ITS2, and LSU), we used the primer pairs ITS1F/ITS4 (White et al. 1990, Gardes & Bruns 1993) and LR0R/LR5 (Vilgalys & Hester 1990, Bunyard et al. 1994).

PCR reactions were carried out under the following thermal cycler conditions for LSU (with modifications for ITS in parentheses): 94°C for 3 min. for initial denaturation; followed by 35 cycles at 94°C for 30 sec. (1 min.), 54°C for 1 min., and 72°C for 1.5 min. (1 min.); then a final extension at 72°C for 4 min. (3 min.). PCR amplicons were then imaged in gel electrophoresis to visualize the presence and relative concentration of fungal DNA. Automated sequencing was carried out at the University of Chicago Comprehensive Cancer Center DNA Sequencing & Genotyping Facility.

Complementary DNA sequences were compiled and manually edited using MEGA 6.0 software suite (Tamura et al. 2013). Additional sequences of closely related taxa from within the *Agaricales* were culled from GenBank (https://www.ncbi.nlm.nih.gov/), with accession numbers cited in the phylogenetic tree (Fig. 2). MEGA 6.0 was used to align sequences and carry out the phylogenetic analysis. A total of 15 LSU nrRNA gene sequences was aligned using the MUSCLE algorithm (gaps were treated as complete deletions), which was used to carry out the maximum likelihood (ML) phylogenetic analysis (NNI heuristic method, with the K2+G nucleotide substitution model chosen under model selection analysis) and bootstrap testing (1000 replicates). The phylogenetic tree was rooted using *Psathyrella candolleana* (*Psathyrellaceae, Agaricales*) as outgroup for *Agaricus* (*Agaricaceae*, *Agaricales*; Matheny 2006).

Taxonomy

Agaricus columellatus (Long) R.M. Chapm., V.S. Evenson & S.T. Bates, **comb. nov.** Figure 1

MycoBank MB 809082

≡ *Araneosa columellata* Long, Mycologia 33(4): 353 (1941).

Type: USA, Arizona, Santa Cruz Co., 7 miles from Nogales on State Highway 89; open areas in mesquite–catclaw (*Prosopis–Acacia*) flats, elev. 3857 ft., 21.IX.1934, William H. Long (7937) and Victor O. Sandberg (the original type collection no. 7937 from Long's Herbarium now exists only as widely distributed isotypes: BPI 736388, BPI 736396, FH 488386, NY 449250!, NY 780610!, NY 780611!, NCU-F-0000798, UBC 371. **Lectotype designated here:** ILL 33529! (Fig. 1E)).

Basidiome 20–35 mm in diameter × 20–30 mm in height, secotioid, subglobose, depressed-globose to broadly ovoid or pyriform, furnished with a rudimentary stipe at the base that extends into the gleba as one unit to form a distinct columella, the entire unit often detaching completely and leaving a circular opening at the base of the peridial body; stipe-columella unit concolorous with the exoperidium, up to 30 mm long, with roughly half (~15 mm) of the unit protruding from the base of the basidiome and the upper half (~15 mm) inserted into the gleba, slightly bulbous at the base, up to 5 mm in diameter and even along the stipe portion, somewhat swollen at the point of insertion, and tapering toward the apex within the gleba as the columella, developing longitudinal striations and becoming more firm when dry, surrounded at the point of insertion by a thin layer of peridial tissue, this with striations radiating out from the central point of the stipe and resembling velar

tissue; PERIDIA slowly breaking apart to expose the gleba, often starting from the basal opening formed as the stipe-columella unit detaches. EXOPERIDIUM whitish to pale yellow (3A1–3), dull, coarsely to irregularly rugose overall, though glabrous at the surface, thin, firm and persistent, cracking apart or flaking off with age to expose portions of the endoperidium. ENDOPERIDIUM yellowish gray (3B2–4B2), dull glabrous, thin, firm and persistent, slowly breaking apart in conjunction with the exoperidium. GLEBA brown to grayish brown (6E3–5), composed of labyrinthiform, sinuous tramal tissue, forming numerous locules, the interior of which is a hymenial surface densely covered with brownish spore mass, and an exterior composed of grayish, loosely woven hyphal tissue, breaking apart into irregular shaped glebal fragments with age.

BASIDIOSPORES 4.0–5.6 × 4.8–7.2 µm [x = 4.8 ± 0.5 × 6.1 ± 0.7 µm, Q_m = 1.3, n = 20], subglobose to ovoid or occasionally irregular ovoid, smooth, brownish in water mounts; pedicel rudimentary or up to 4 µm in length; STERIGMATAL REMNANTS mostly absent from mounts. CAPILLITIUM absent. PERIDIA composed of thin-walled hyphal elements.

HABITAT: Terrestrial and found in soil amongst *Acacia*, palo verde (*Parkinsonia*), creosote (*Larrea*), mesquite (*Prosopis*) and jojoba (*Simmondsia*). *Agaricus columellatus* occurs at lower elevations within the Arizona Upland Subdivision of the Sonoran Desertscrub and Semidesert Grassland biotic communities (Brown 1994).

DISTRIBUTION: In the United States, known only from southern Arizona. Also reported from the state of Sonora in Mexico.

GENETIC DATA: Nuclear ribosomal RNA gene sequences for *Agaricus columellatus* (MIN 938394) have been deposited in GenBank (ITS: KJ912899; LSU: KJ912900).

ADDITIONAL SPECIMENS EXAMINED: **USA**, **ARIZONA**, GILA CO., AZ 288, north of Globe, 33.67°N 110.96°W, alt. 707 m, in sand, amongst *Acacia*, palo verde, creosote bush, and jojoba, 30.VIII.2012, R.M. Chapman 1385 (MIN 938394); AZ 288, north of Globe, 33.67°N 110.97°W, alt. 721 m, in sand, amongst acacia, palo verde, creosote bush, and jojoba, 1.IX.2012, R.M. Chapman 1388 (hb. Chapman). SANTA CRUZ CO., 7 miles north of Nogales, Arizona on Nogales–Tucson road, 30.IX.1939, William H. Long 8388 (paratype NY 2169336).

Results & discussion

Long (1941) described *Araneosa columellata* within *Arachniaceae*, stating "peridioles subglobose to irregularly oblong to angular, walls rather thick and firm, 90–268 microns by 134–402 microns, held loosely together by delicate interwoven hyphae, easily separable from the endoperidium, leaving its inner surface reticulate, the lamelliform arrangement of the peridioles often permitting the gleba to split into flakes which easily crumble into the

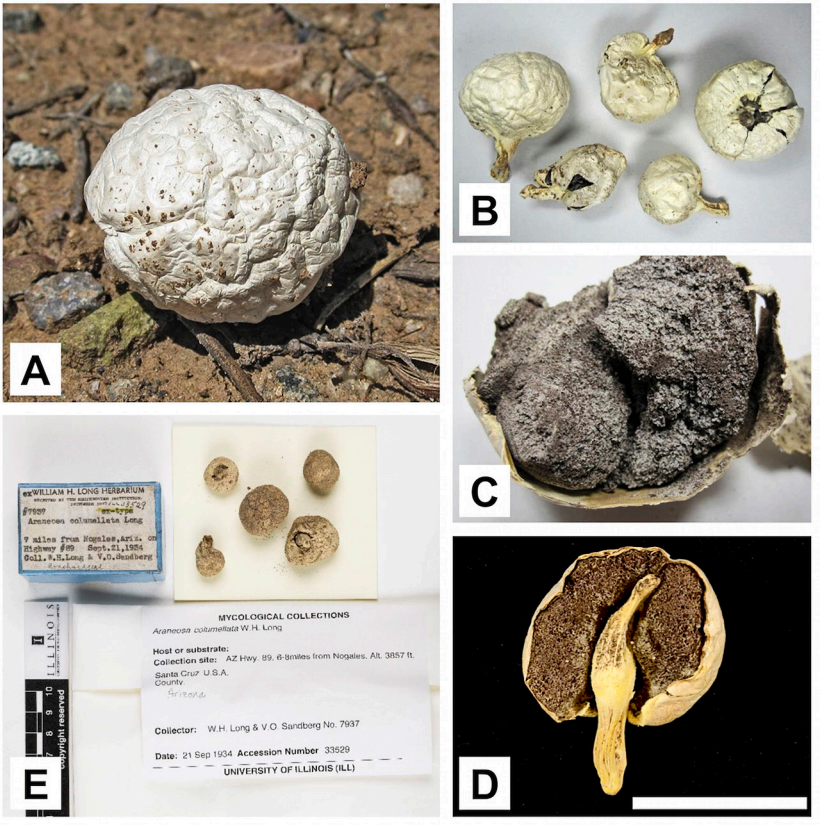

Figure 1. *Agaricus columellatus*. A. Field image of the gasterocarp (MIN 938394). B. Dried specimens (MIN 938394, bar = 20 mm). C. Exposed gleba of dried specimen (MIN 938394). D. Cross section through basidiome of dried specimen showing insertion of stipe-columella unit (MIN 938394; bar = 20 mm). E. Lectotype (ILL 33529).

individual peridioles." Although our material was morphologically identical to Long's (1941) concept of *Ar. columellata*, distinct peridioles did not form in the gleba. Inspection of authentic material from Long (NY 449250, NY 780610, NY 780611, NY 2169336, and the lectotype ILL 33529), revealed in these specimens the formation of irregularly shaped glebal fragments rather than discrete peridioles, which is consistent with our material (Fig. 1C). While the age of these specimens prevented us from confirming an exact match to the Long specimens by molecular means, on the basis of morphology, our specimens clearly represent a recent collection of *Ar. columellata*.

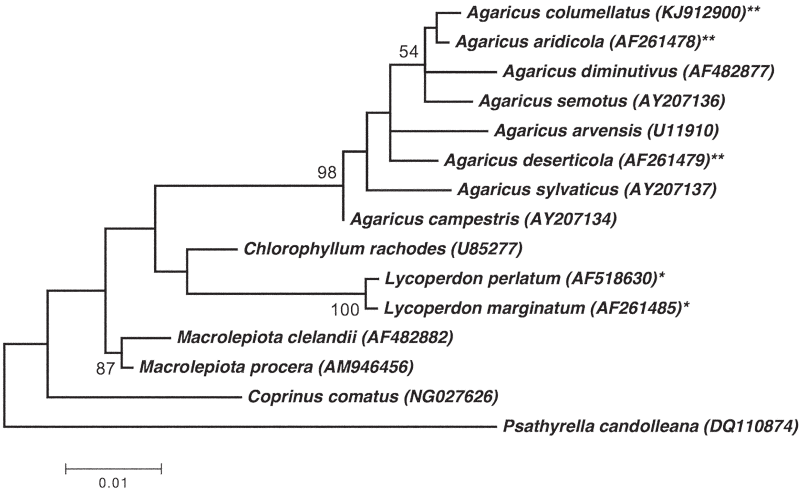

Figure 2. Molecular phylogeny (ML consensus tree) for the large subunit nuclear ribosomal RNA gene dataset showing the inclusion of *Agaricus columellatus* (MIN 938394), within *Agaricus* (*Agaricaceae*). Basidiome forms are agaricoid, unless otherwise indicated as gastroid (*) or secotioid (**). Branch lengths are shown at scale, with the bar representing the number of base pair changes along the branches, and nodes with ≥50% bootstrap support are shown. The tree is rooted with *Psathyrella candolleana* (*Psathyrellaceae*), and the GenBank accession numbers are given for all taxa included in the tree.

Phylogenetic analysis of the LSU nrRNA gene alignment places our collection of *Ar. columellata* within *Agaricus* (*Agaricaceae*; Fig. 2); therefore, we transfer *Araneosa columellata* to this genus here as *Agaricus columellatus*. While our LSU tree (Fig. 2) shows *Ag. columellatus* as sister to *Ag. aridicola* (≡ *Gyrophragmium dunalii*), ITS sequence data (tree not shown) do not support a sister relationship for these two taxa, which share only 95% sequence similarity in the ITS region. Of the several Australian secotioid *Agaricus* species reported in the study of Lebel & Syme (2012), *Ag. columellatus* was most closely related to *Ag. wariatodes;* however, ITS sequence similarity between these two taxa was likewise very low (95%). Thus our study suggests yet another example of the independent origin of a unique secotioid fruiting body within the genus *Agaricus* and of the angiocarpic form more broadly across the *Agaricaceae*.

The distribution of *Ag. columellatus* is restricted to the desert regions of the southwestern U.S.A. and northern Mexico, with reports from the environs of Tucson and Nogales, Arizona, as well as Hermosillo in Sonora, Mexico (Long 1941, Esqueda et al. 1998, Moreno et al. 2007). The recorded range extends

a linear distance of approximately 400 km (north to south), while this study expands the distribution roughly another 170 km northward to the area of Globe, Arizona. This distribution essentially spans the western edge of the Sonoran Desert, though the area surrounding Nogales is technically considered outside of its boundary (Brown 1994). More extensive collecting in the area will almost certainly extend the range westward within the Sonoran Desert, and potentially eastward into the Chihuahuan Desert. The occurrence of *Ag. columellatus* within the arid-lands of North America reinforces the concept that selective pressure of xeric climates may have driven the evolution of angiocarpic forms from their agaricoid ancestors (Thiers 1984, Lebel & Syme 2012).

Acknowledgments

Specimen data were provided by institutions participating in the Macrofungi Collections Consortium (NSF ADBC 1206197) project, which have been made available through the Mycology Collections data Portal (http://mycoportal.org). The authors thank Drs Bradley R. Kropp (Utah State University, Logan) and Else C. Vellinga (University of California, Berkeley) for presubmission review.

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